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Gene-centric Meta-analysis in 87,736 Individuals of European Ancestry Identifies Multiple Blood-Pressure-Related Loci

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Blood pressure (BP) is a heritable risk factor for cardiovascular disease. To investigate genetic associations with systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), and pulse pressure (PP), we genotyped ~50,000 SNPs in up to 87,736 individuals of European ancestry and combined these in a meta-analysis. We replicated findings in an independent set of 68,368 individuals of European ancestry. Our analyses identified 11 previously undescribed associations in independent loci containing 31 genes including *PDE1A*, *HLA-DQB1*, *CDK6*, *PRKAG2*, *VCL*, *H19*, *NUCB2*, *RELA*, *HOXC@ complex*, *FBN1*, and *NFAT5* at the Bonferroni-corrected array-wide significance threshold ($p < 6 \times 10^{-7}$) and confirmed 27 previously reported associations. Bioinformatic analysis of the 11 loci provided support for a putative role in hypertension of several genes, such as *CDK6* and *NUCB2*. Analysis of potential pharmacological targets in databases of small molecules showed that ten of the genes are predicted to be a target for small molecules. In summary, we identified previously unknown loci associated with BP. Our findings extend our understanding of genes involved in BP regulation, which may provide new targets for therapeutic intervention or drug response stratification.

Introduction

Blood pressure (BP) is a major, modifiable determinant of cardiovascular disease (CVD) risk.[1] Hypertension (MIM 145500) increases risk for a variety of sequelae, including coronary artery disease, heart failure (MIM 608320), stroke (MIM 601367), and peripheral vascular disease (MIM 606787).[2] Heritability of daytime ambulatory BP from

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twin studies has been estimated to be approximately 50%–60%[3,4] and ~40% in family studies. Several studies have been performed to elucidate underlying genetic factors for BP, including genome-wide association studies (GWASs), meta-analyses, and admixture mapping.[5–12] Efforts to dissect the genetic basis for this complex disorder have proven challenging and currently only a small portion of the total variation in BP can be explained by common genetic variants associated signals. Part of this "missing heritability" is likely to be due to as yet unknown common or low-frequency variants, and a fraction of it could be identified by increasing sample size. Increased understanding of the underlying genetics may contribute to improvement of the treatment to reduce CVD risk.

We conducted an association analysis of BP phenotypes in 87,736 individuals of European ancestry by using a gene-centric array with ~50,000 SNPs capturing variation in ~2,100 genes related to CVD, and we replicate our findings in 68,368 independent individuals. We also performed extensive bioinformatics analyses to identify regulatory regions by using the Encyclopedia of DNA Elements (ENCODE), eQTL studies, and pathway analyses. Finally, all previously reported BP-associated loci and the genes within were annotated for their suitability as drug targets.

Material and Methods

Population Characteristics

Phenotype and genotype data from 87,736 individuals of European ancestry from 36 participating studies were used for the discovery phase, and an additional 68,368 individuals of European ancestry, from 19 additional studies, were used in the replication phase. All individuals in these studies provided informed consent, and each study was approved by its own local ethics committee. Descriptions of the participating cohorts are provided in the original publications.[5,8,11,13]

Phenotypes

Pulse pressure (PP) was defined as systolic blood pressure (SBP) minus diastolic blood pressure (DBP), and median arterial pressure (MAP) was defined as 2/3 DBP plus 1/3 SBP. Each cohort provided regression models for its respective data, adjusted for age, age-squared, body mass index (BMI), and any study-specific corrections for population substructure (based on principal components analysis). BP values were adjusted for antihypertensive drug therapy by adding a standard treatment adjustment of 15 mmHg to the SBP and 10 mmHg to the DBP values of individuals receiving treatment in the discovery and replication cohorts. These adjustments were implemented prior to the calculation of MAP and PP.

Genotyping

A total of 52,029 SNPs were included in the meta-analyses, and all SNPs were present in at least one of the three iterative versions of the Illumina HumanCVD BeadChip (also known as the "Cardiochip" or the ITMAT-Broad-CARe [IBC] array manufactured by Illumina)[14] used by all discovery cohorts. Several studies that used this array have already been published for a variety of phenotypes and disease outcomes, including coronary artery disease,[16,17] plasma lipids,[18] BP,[8,11,15] heart failure,[19] type 2 diabetes (T2D [MIM 125853]),[20] and BMI (MIM 606641).[21]

Quality Control, Association, and Meta Analyses

The discovery data sets derive from three BP consortia with completely independent samples whose results have been published separately: the IBC BP consortium[8] and the CVD-50 consortium,[22] with the addition of the Beaver Dam Studies (BOSS, EHLS, BDES) consortium.[13] The QC steps taken for SNPs and samples were similar for both consortia, namely: individuals with <90% call rate (completeness) across all SNPs were removed, as well as SNPs with <95% call rate (completeness) or SNPs causing heterozygous haploid genotype calls across all remaining individuals. SNPs were also removed if they were not in Hardy-Weinberg equilibrium ($p < 1 \times 10^{-7}$). No study included imputed data in their analyses. Further details on genotyping and QC can be found in the original publications including the BOSS, EHLS, and BDES studies.[8,11,13]

During the meta-analysis step, SNPs with frequencies incompatible with HapMap CEU frequencies were removed (defined as >30% difference in the minor allele frequencies) for each data source separately, i.e., the meta-analysis results of IBC BP, of CVD-50, and of BOSS, EHLS, and BDES. We used inverse variance weighted meta-analysis in MANTEL[23] to obtain a fixed-effect estimate and statistical significance for each SNP. We applied genomic control[24] to each data set to control effects possibly resulting from population stratification or cryptic relatedness. The CARe IBC array studies, included in this meta-analysis, determined that after accounting for linkage disequilibrium (LD), the effective number of independent tests was ~20,500 for Europeans. This resulted in an experimental or "arraywide" statistical threshold of $p = 2.4 \times 10^{-6}$ to maintain a false-positive rate of 5%[25] and therefore we have adopted this threshold for this study. For each associated locus, the LD patterns were examined and independence between the loci identified in this study and previously published signals was verified with SNAP[26] ($r^2 < 0.3$).

Replication analysis was conducted in independent samples for each trait, for SNPs with association $p < 1 \times 10^{-5}$ in the discovery analysis, with a total of 68,368 individuals from the Global Blood Pressure Genetics (GBPG) consortium,[10] Women's Genome Health Study (WGHS),[27] and PREVEND[28] and LifeLines[29] studies. We combined discovery and replication data in a meta-analysis and accounted for testing four phenotypes (albeit highly correlated), resulting in a Bonferroni-corrected threshold of $p < 6 \times 10^{-7}$ for the combined meta-analysis of discovery and replication samples.

Definition of Associated Gene Variants and Variant Functional Analysis

The extended locus around each associated SNP was defined by identification of all SNPs showing $r^2 \geq 0.5$. Linkage disequilibrium was defined with the HaploReg tool[30] based on Phase I of the 1000 Genomes project. Variants showing evidence of LD with associated variants were explored for impact on gene function via Annovar[31] and regulatory function (including eQTLs) by HaploReg[30] and RegulomeDB,[32] which both collate data from the Encyclopedia of DNA Elements (ENCODE)[33] and nine eQTL studies.[32] Associated genes were reviewed for evidence linking them to BP-related phenotypes via PubMed and "hypertension," "cardiovascular disease," or "vascular disease" as medical subject headings (MeSH) terms. MeSH is a controlled vocabulary created

by the National Library of Medicine (NLM) to index journal articles and books in the life sciences. All articles in MEDLINE have been annotated with MeSH by NLM curators or designees, offering a sensitive measure of correlation between biological traits and genes in the literature.[34] We also checked for annotation to the gene ontology term "regulation of blood pressure," which also represents a highly curated set of genes consistently linked to blood pressure.[35] At a pathway level, we used GeneGo Metacore (Thomson Reuters) to construct a custom BP network based on 436 genes annotated to the "hypertension" MeSH term and gene ontology terms described above. The Metacore database is a large commercial database of curated human, rat, and mouse gene and protein interactions individually evidenced in the literature.[36] This allowed us to construct a core network of interacting genes that are each individually linked to blood pressure, which collectively are likely to represent a significant blood pressure gene network. We used this network as a tool to investigate direct interactions between the blood pressure gene network and the genes in the blood pressure loci reported here. By combining network data with data on gene and variant function, we were able to prioritize genes based on their level of support with respect to BP phenotypes.

Analysis of Pharmacologic Targets

We annotated genes containing variants in LD (HapMap CEU $r^2 > 0.5$) with discovered associations and analyzed information concerning potential druggability—that is, the potential for modulation of the protein target by a water-soluble small-molecule drug. Druggable proteins usually contain a defined binding pocket or active site, which could act as a site of action (pharmacophore) for an orally bioavailable small-molecule drug. We grouped proteins into four druggability classes, based on complementary annotations of the potentially druggable genome and publicly available databases of small molecules. Targets in class 1 are already drugged with a marketed drug recorded in DrugBank; class 2 have small molecules recorded in ChEMBL, which may include compounds in current development within pharmaceutical companies, and could be used as tools in animal and cellular models; class 3 are homologous to class 1 or class 2 targets; and class 4 are predicted to contain a potentially druggable pharmacophore based on de novo structure-based druggability prediction via the online available DoGSiteScorer tool,[37] which binds site prediction, analysis, and druggability.

Expression Quantitative Trait Loci Analysis

We identified alias identifiers for significant index SNPs by using SNAP, an online tool for LD calculations.[26] Further proxy SNPs displaying high LD ($r^2 > 0.8$) were identified across four HapMap builds in European ancestry samples (CEU) with SNAP. The primary SNPs and LD proxies were searched against a collated database of expression SNP (eSNP) results including the following tissues: fresh lymphocytes,[38] fresh leukocytes,[39] leukocyte samples in individuals with celiac disease (MIM 212750),[24] whole blood samples,[40–43] lymphoblastoid cell lines (LCLs) derived from asthmatic children (MIM 600807),[44,45] HapMap LCLs from three populations,[46] a separate study on HapMap CEU LCLs,[47] additional LCL population samples,[48–51] $CD19^+$ B cells,[52] primary PHA-stimulated T cells,[48] $CD4^+$ T cells,[53] peripheral blood monocytes,[52,54,55] $CD11^+$ dendritic cells before and after *Mycobacterium tuberculosis* infection (MIM 607948),[56] omental and subcutaneous adipose,[40,50,57] stomach,[57] endometrial carcinomas (MIM 608089),[58] ER^+ and ER^- breast cancer tumor cells (MIM 114480),[59] brain cortex,[54,60,61] prefrontal cortex,[62,63] frontal cortex,[64] temporal cortex,[61,64] pons,[64] cerebellum,[61,64] three additional large studies of brain regions including prefrontal cortex, visual cortex, and cerebellum,[65] liver,[57,66,67] osteoblasts,[68] ileum,[57,69] lung,[70] skin,[50,71] and primary fibroblasts.[48] MicroRNA QTLs were also queried for LCLs[72] and gluteal and abdominal adipose.[73] The collected eSNP results met the criteria for association with gene expression levels as defined in the original papers. The majority of eQTLs (15/17) showed a p value of 1×10^{-6} or less. Two replication studies with more modest association were also included. We placed more value on eQTLs with low p values that showed consistent reporting across independent studies. In each case where a SNP or proxy was associated with transcript levels, we further examined the strongest eSNP for that transcript within that data set (best eSNP) and the LD between the best eSNP and BP-selected eSNPs to estimate the concordance of the BP and expression signals.

Results

Discovery Meta-analysis

In the discovery meta-analysis, four BP traits were analyzed in 87,736 individuals from 36 cohorts, as described in Table S1 available online. We analyzed SBP, DBP, MAP, and PP as continuous traits. Cohort characteristics, including age, sex, BP values, and the proportion of individuals treated with BP-lowering medications, are provided in Table S1.

Association analyses were successfully carried out for up to 48,616 SNPs that passed QC. We identified 17 SNPs that passed a suggestive discovery p value threshold of $p < 1 \times 10^{-5}$ with six SNPs showing strongest associations with SBP, i.e., lowest p value among all four traits (and one secondary association, i.e., a threshold-passing p value but with a higher p value than in another trait), two SNPs showing strongest associations with DBP (and two other secondary associations), two showing strongest associations with MAP (and two other secondary ones), and three leading associations to PP (with three secondary associations).

Replication Analyses

Replication testing was performed in 68,368 additional individuals from 19 cohorts with genome-wide SNP genotypes imputed to HapMap, only to the signals passing the threshold on discovery and previously not published. A meta-analysis of the 17 SNPs taken forward from the discovery phase with replication data showed that 11 SNPs at independent loci met our Bonferroni-corrected array-wide significance threshold of $p < 6 \times 10^{-7}$. Some of these SNPs showed association with more than one trait, which resulted in 17 previously not described associations: three loci were associated with DBP (*PDE1A* [MIM 171890], *HLA-DQB1* [MIM 604305], and *VCL* [MIM 193065]), five loci were associated with SBP (*PRKAG2* [MIM 602743], *H19* [MIM 103280], *NUCB2* [MIM 608020], *SIPA1* [MIM 602180], and *HOXC* complex [MIM 142974]), five loci

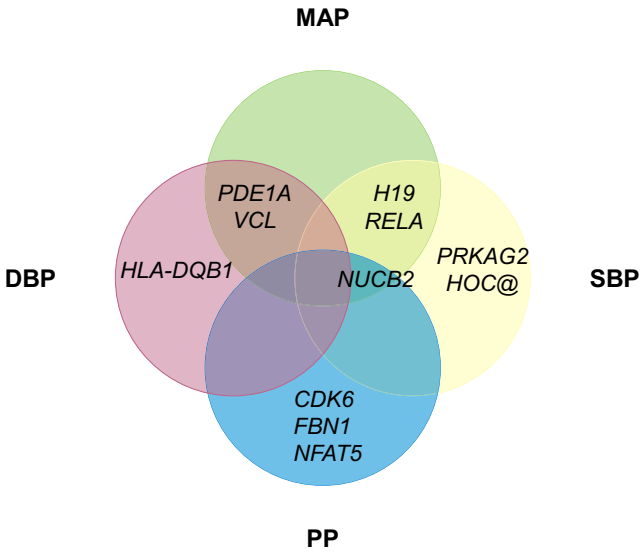

Figure 1. Overview of the Replicated Blood-Pressure-Related Findings from This Meta-analysis for Overlap with the Various Blood-Pressure-Related Traits
Abbreviations are as follows: SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; and PP, pulse pressure.

were associated with MAP (*PDE1A*, *VCL*, *H19*, *NUCB2*, and *RELA* [MIM 164014]), and four loci were associated with PP (*CDK6* [MIM 603368], *NUCB2*, *FBN1* [MIM 134797], and *NFAT5* [MIM 604708]) (Figure 1). The association results are summarized in Table 1. We also found a suggestive association in *ERAP1* (MIM 606832) with $2.4 \times 10^{-6} > p > 6 \times 10^{-7}$, which reached array-wide significance but not Bonferroni-corrected significance (array-wide significance divided by the number of traits, four).

We compared the results of our analysis with all published associations at the time of this report (to the best of our knowledge)[5,8–11,22,74–77] and confirmed previously reported BP associations at 27 loci with same direction of effect (at a nominal association threshold [$p < 0.05$]), out of 32 loci covered by this genotyping array, with partial sample overlap between original findings and our study (Table S2 contains all previously reported loci and our p values for these associations; each column with an X indicates a significant result was found by the referred study to the specified trait). We did not find supportive evidence of association with BP of five loci: *CACNB2* (MIM 600003), *CDH13* (MIM 601364), *C10orf107* (MIM not available), *ZNF652* (MIM 613907), and *STK39* (MIM 607648), which were found in GWAS meta-analyses. The IBC array does not contain lead SNPs or proxies for 18 of the previously reported loci identified by GWASs.

eQTLs

Several of the reported loci have significant eQTLs. rs2854275 is associated with the expression of *HLA-DRB4* in monocytes ($p = 4.82 \times 10^{-95}$) and in liver ($p = 1.31 \times 10^{-10}$) and *HLA-DRB1* (MIM 142857) in blood ($p = 3.20 \times 10^{-59}$); rs2282978 associates with *CDK6* expression (MIM 603368) ($p = 2.6 \times 10^{-5}$); rs4746172 is in perfect LD with rs10824069, which presents a significant association with expression of the ADK transcript (MIM 102750) in lung ($p < 2 \times 10^{-16}$); and rs217727 is associated with increased expression of AK126915 in LCLs ($p = 2.40 \times 10^{-6}$) and skin ($p = 6.59 \times 10^{-6}$). Closer investigation of the AK126915 transcript in the UCSC Genome Browser suggests that it may represent an isoform of the mitochondrial ribosomal protein L23 (*MRPL23* [MIM 600789]) (data not shown). rs757081 is associated with expression of *NUCB2* (MIM 608020) in LCLs in asthmatics ($p = 1.56 \times 10^{-16}$) and of *SNORD14A* in LCLs ($p = 3.10 \times 10^{-5}$); rs33063 is associated with expression of another unknown transcript, LOC283970 in B cells ($p = 9.55 \times 10^{-8}$); and rs3741378 is in tight LD with several SNPs that present significant eQTL levels in lymph, liver, and other tissues. Table S4 presents the results in more detail.

Extended Locus Analysis and Variant Functional Analysis

Variants in LD ($r^2 > 0.5$) with the 11 replicated SNPs are functionally annotated in Table S5, and a nonredundant list of corresponding genes appear in Table S6. Several loci contained only one gene (*PDE1A*, *HLA-DQB1*, *CDK6*, *PRKAG2*, and *FBN1*), whereas others contained several genes (see Table S6). By comprehensive functional annotation, we reviewed the genes at each locus both for evidence of functional impact (genic and regulatory) and a rationale in BP.

Discussion

In this study we identified 11 loci associated with BP traits ($p < 6 \times 10^{-7}$) in a meta-analysis comprising 87,736 individuals of European descent. These associations were validated in a replication cohort of 68,368 individuals. The robust sample size also confirmed a nominal association ($p < 0.05$) of 27 previously reported signals, out of the 32 previously reported loci covered by this array.

Associated Loci with a Single Candidate Gene

Of the 11 loci described in this study, 5 contain only a single gene. The PDE1A locus contains the phosphodiesterase 1A (*PDE1A*) gene, a Ca^{2+}/calmodulin-stimulated phosphodiesterase that preferentially hydrolyzes cGMP and has an important role in regulating vascular tone and smooth muscle cell proliferation.[78,79] The HLA-DQB1 locus contains *HLA-DQB1* (major histocompatibility complex, class II, DQ beta 1), which encodes a class II molecule expressed in antigen-presenting cells and plays a role in the immune system by presenting peptides derived from extracellular proteins. *HLA-DQB1* alleles have been linked to essential hypertension in Chinese populations.[80] At the PRKAG2 locus, *PRKAG2* (protein kinase, AMP-activated, gamma 2 noncatalytic subunit) is

Table 1. Significant Association Results for All Four Traits in the Meta-analysis

Locus	SNP	CHR	Pos	Effect Allele	Discovery p Values (n = 87,736)	Replication p Values (n = 68,368)	Combined Discovery + Replication beta	SE	p Val
DBP									
PDE1A	rs16823124	2	182932372	A	1.76×10^{-6}	2.59×10^{-5}	0.262	0.041	1.95×10^{-10}
HLA-DQB1	rs2854275	6	32736406	A	2.97×10^{-8}	1.86×10^{-4}	−0.562	0.101	5.53×10^{-12}
VCL	rs4746172	10	75525848	C	2.14×10^{-5}	1.07×10^{-3}	0.230	0.043	9.14×10^{-8}
MAP									
PDE1A	rs16823124	2	182932372	A	1.10×10^{-4}	3.85×10^{-4}	0.269	0.052	1.90×10^{-7}
VCL	rs4746172	10	75525848	C	7.91×10^{-6}	8.73×10^{-3}	0.279	0.054	2.45×10^{-7}
H19	rs217727	11	1973484	A	1.63×10^{-5}	3.94×10^{-3}	0.315	0.061	2.15×10^{-7}
NUCB2	rs757081	11	17308259	G	3.23×10^{-5}	1.16×10^{-3}	0.265	0.050	1.39×10^{-7}
RELA	rs3741378	11	65165513	T	3.08×10^{-5}	2.30×10^{-3}	−0.359	0.070	2.44×10^{-7}
PP									
CDK6	rs2282978	7	92102346	C	3.82×10^{-8}	0.073	−0.268	0.049	4.51×10^{-8}
NUCB2	rs757081	11	17308259	G	4.51×10^{-7}	4.48×10^{-5}	0.321	0.050	8.94×10^{-11}
FBN1	rs1036477	15	46702218	G	6.59×10^{-6}	8.02×10^{-3}	−0.402	0.077	2.00×10^{-7}
NFAT5	rs33063	16	68197718	A	9.07×10^{-7}	0.063	0.335	0.066	4.14×10^{-7}
SBP									
PRKAG2	rs10224002	7	151045974	G	1.67×10^{-6}	0.023	0.361	0.072	5.50×10^{-7}
H19	rs217727	11	1973484	A	7.88×10^{-6}	2.95×10^{-3}	0.417	0.078	1.03×10^{-7}
NUCB2	rs757081	11	17308259	G	2.26×10^{-7}	1.28×10^{-4}	0.403	0.063	1.65×10^{-10}
RELA	rs3741378	11	65165513	T	1.70×10^{-6}	4.42×10^{-5}	−0.546	0.087	3.41×10^{-10}
HOXC@	rs7297416	12	52729357	C	2.26×10^{-6}	0.011	−0.334	0.065	2.32×10^{-7}

important in cellular metabolism and has been associated with Wolff-Parkinson-White syndrome (MIM 194200), urate levels and chronic kidney disease,[81,82] and accumulation of cardiac glycogen and left ventricular hypertrophy resembling hypertrophic cardiomyopathy.[83,84] Chronic kidney disease and left ventricular hypertrophy represent target organ damage related to hypertension, and thus, our findings may provide a link between genetic loci affecting BP and the occurrence of clinically important sequelae. Other traits associated with *PRKAG2* include hemoglobin[85] and hematocrits.[86] The FBN1 locus encodes fibrillin-1 (*FBN1*), a component of elastic fibers in connective tissue, and this gene has been associated with Marfan syndrome (MIM 154700), with systolic and pulse pressure, with aortic stiffness in patients with coronary artery disease (CAD),[87,88] and with thoracic aortic aneurysms and thoracic aortic dissection.[89] *CDK6* variants are associated with height (MIM 606255) in a number of studies[90–92] and are implicated in white blood cell counts in Japanese populations[93,94] and in African Americans.[95]

Associated Loci with Multiple Genes

The remaining loci (VCL, H19, NUCB2, SIPA1, HOXC4, and NFAT5) contain several genes that we have prioritized on the basis of variant functionality and the biological rationale of the genes in the BP phenotypes.

We consider vinculin (*VCL*) the strongest biological candidate. Vinculin is a cytoskeletal protein, localized to intercalated discs, and by anchoring thin filaments it is implicated in cardiac force generation. Targeted disruption of vinculin in mice has shown loss of cardiac contractility in embryonic development.[96] The two other candidates at the VCL locus (*AP3M1* and *ADK*) show moderate evidence of functional variation.

The H19 locus contains three genes. *H19* (H19, imprinted maternally expressed transcript [nonprotein coding]) expresses a noncoding RNA. Methylation defects in this gene have been associated with pre-eclampsia in a study with 188 pregnancies[97] and imprinting syndromes such as Beckwith-Wiedemann syndrome (MIM 130650) and growth retardation disorder Silver-Russell syndrome (MIM 180860).[98,99] The locus also contains the mitochondrial ribosomal protein L23 (*MRPL23*) and an antisense transcript MRPL23-AS1. There are no data suggesting a putative role in BP for the latter, but eQTL analysis identified a putative isoform of *MRPL23* (AK126915).

The NUCB2 locus contains four genes: *PIK3C2A*, *NUCB2*, *NCR3LG1*, and *KCNJ11*. The product of *NUCB2*,

nucleobindin 2, induces hypertension when intracerebrally administered.[100] It is also involved in the maintenance of calcium blood levels, feeding behavior, water intake, glucose homeostasis, and the release of tumor necrosis factor from vascular endothelial cells by interacting with *ERAP1*.[101,102] It has been also associated with height in GWASs.[90–92] The potassium inwardly rectifying channel, encoded by *KCNJ11*, is also a good biological candidate, which has been reported to be associated with hypertension[103] as well as type 2 diabetes, although the previously reported variants are not in LD with the association reported in this study.

The SIPA1 locus contains seven genes: *MAP3K11*, *PCNXL3*, *SIPA1*, *RELA*, *KAT5*, *RNASEH2C*, and *AP5B1*. *SIPA1* (signal-induced proliferation-associated 1) is the strongest functional candidate, and the associated SNP encodes a nonsynonymous p.Ser182Phe polymorphism, which is predicted to be deleterious by several bioinformatics tools.[31] The strongest biological candidate is the adjacent *RELA* (a.k.a. NF-κB3), which forms part of the NF-κB protein complex and has been shown to modulate angiotensin II-induced hypertension in the paraventricular nucleus.[104]

The HOXC4 locus contains three homeobox genes: *HOXC4*, *HOXC5*, and *HOXC6*. All three genes are closely related and encode transcription factors involved in morphogenesis. A recent trans-ethnic GWAS on blood pressure found a signal related to HOXCA,[105] suggesting that homeoboxes may have an association with blood pressure in more ethnicities.

The NFAT5 locus also contains several candidates, including *NFAT5* (Nuclear factor of activated T cells 5). *NFAT5* is a transcription factor, recently shown to regulate vascular smooth muscle cell (VSMC) modulation.[106] In vivo studies in $NFAT5^{+/-}ApoE^{-/-}$ mice indicated that NFAT5 is directly involved in atherosclerotic lesion formation and identified NFAT5 as a positive regulator of atherosclerotic lesion formation.[107] A variant at this locus has also been associated with serum urate[81] and age at menarche.[108] Small-molecule activators of the adjacent *NQO1* (NAD(P)H:quinone oxidoreductase) have been shown to ameliorate spontaneous hypertension in animal models via modulation of eNOS activity.[109] The Ubiquitin-protein ligase *WWP2* is also a candidate, because it is known to bind and downregulate the epithelial Na(+) channel (ENaC). Mutations in *WWP2* have been shown to result in hypertension.[110]

Therapeutic Opportunities

Current antihypertensive medications do not show complete efficacy in all subjects and often require combination therapy of three or more drugs to reach a target blood pressure, and ~10% of individuals show limited reduction in blood pressure on all therapeutic regimens.[111] This highlights the need for additional antihypertensive medications, both to improve efficacy of treatment and reduce the burden of side effects experienced, particularly where combination therapies are concerned. Among the associations reported in this manuscript, there is evidence of association for 15 genes that are either current drug targets or are potentially druggable based on the predicted potential of a protein to be modified by a small-molecule drug in our analyses. Two genes are targeted by currently widely applied drugs. The *KCNJ11* product is targeted by several agents including the antihypertensive drug verapamil (DrugBank ID DB00661) and the glucose-lowering agent glyburide (DrugBank DB01016). NQO1 is targeted by several marketed anticoagulant drugs, including dicumarol and menadione (DrugBank DB00266 and DB00170). The protein encoded by *RELA* is part of the NF-κB protein complex, which is considered to be inhibited by several known drugs, including the antihypertensive olmesartan (DrugBank DB00275)[112] and the alcohol deterrent drug disulfiram (DrugBank DB00822). A known side effect of disulfiram in presence of alcohol exposure is hypotension, which gives support to a potential role of *RELA* in hypertension.

Nine genes with evidence of association have also previously described small-molecule modulators (mainly inhibitory or binding) based on a query of the ChEMBL database. The genes are listed with the number of tool compounds in parentheses: *PDE1A* (42), *CDK6* (405), *ADK* (483), *MAP3K11* (192), *RELA* (289), *KAT5* (25), and *PIK3C2A* (10). The number of molecules identified may indicate the extent of drug discovery research that has been focused on each target. Many are likely to be the focus of pre-existing drug development in industry that may still be ongoing or terminated.[113] Once molecular properties of these compounds are considered to have a favorable profile, they could be investigated in animal models of hypertension; a large number of compounds have already been characterized: the ChEMBL database reports that 405 molecules show activity against CDK6. Most of these CDK6 inhibitors originate from the published GSK (Glaxosmithkline) kinase inhibitor set.[113] A review of the molecular properties of these molecules in ChEMBL shows that they have similar drug-like features and are likely to be orally bioavailable (based on Lipinski's rule of five compliance[114]). As we described earlier, association is limited to *CDK6* only, and because many of these drug-like molecules would be suitable for immediate evaluation in animal models of hypertension, this might be a worthwhile experiment, even in the absence of other strong evidence to support the role of CDK6 in BP.

The 11 loci identified in the present study increase our knowledge on BP-related processes and shed light on plausible candidates at each locus and networks, via eQTL and pathway analysis. It is particularly interesting that most of the genes within the associations are suitable candidates for existing drugs or preclinical compounds. These new observations will help to improve our knowledge on BP and related mechanisms.

Supplemental Data

Supplemental Data include Acknowledgments and six tables and can be found with this article online at http://www.cell.com/AJHG/.

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Web Resources

The URLs for data presented herein are as follows:

ChEMBL, http://www.ebi.ac.uk/chembl
DrugBank, http://www.drugbank.ca
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
SNP Annotation and Proxy Search (SNAP), http://www.broadinstitute.org/mpg/snap/
UCSC Genome Browser, http://genome.ucsc.edu

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